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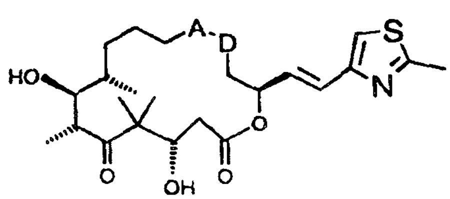

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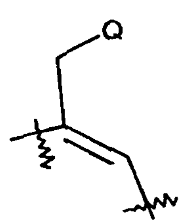

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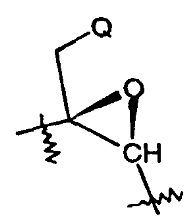

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(57) Abstract

The invention relates to compounds of formula (I), wherein the group A–D represents a group of formula (Ia) or of formula (Ib) wherein Q is hydroxy, iodo or hydrogen, and in addition to methods of synthesis for these compounds, as well as for the synthesis of epothilone B, and intermediates. The compounds of formula (I) can be used e.g. in the treatment of proliferative diseases.

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Desmethyl Epothilones

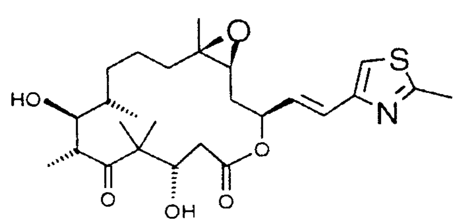
Summary of the invention

The present invention relates to a new method of synthesis of epothilone B and epothilone B analogs that is highly selective at every step and increases the speed of access to epothilone B and epothilone B analogs. It also relates to 16-desmethyl analogues of epothilone B and its deoxy form, methods for producing such compounds, their use in the therapy of diseases or for the manufacture of pharmaceutical preparations for the treatment of diseases, as well as to novel intermediates used in the synthesis of said compounds.

Background of the invention

During synthesis of 16-desmethyl epothilone B, also new methods for the convergent and highly stereoselective synthesis of epothilone B and analogues were developed.

With their paclitaxel-like mechanism of action, epothilones have recently been at the focus of widespread scientific investigations. Particularly, the chemical synthesis of the epothilone natural products has been successfully achieved (see, e.g., Nicolaou et al., Angew. Chem. Int Ed. 37, 2014-2045 (1998); May et al., Chem. Commun. 1998, 1597-8, Schinzer et al., Synlett. 1998, 861-4; Balog et al., Angew. Chem. Int. Ed. 37, 2675-78 (1998); Mulzer et al., Tetrahedron Let.. 39, 8633-6 (1998); or Sinha et al., Proc. Natl. Acad. Sci. USA 95, 14603-14608 (1998)). In connection with ongoing studies on structure-activity relationships in order to find epothilone congeners with improved pharmacological profiles, we were interested in the preparation of analogues of epothilone B with less conformationally restrained heteroaromatic side-chains, in particular the 16-desmethyl analogue of the formula A:



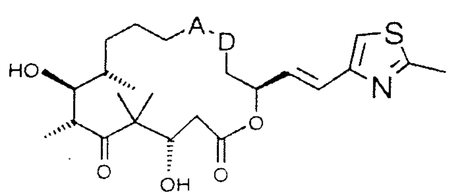
(A)

This required complete re-synthesis of the epothilone macrocycle and thus was an opportunity to develop new methods with improved selectivity and yields for the swift assembly of epothilone B analogues.

Based on the highly modular and convergent approach to epothilone A and analogues via olefin metathesis (see Nicolaou et al., loc. cit.), we envisioned that our approach to epothilone B analogues could be made more convergent by employing a fully functionalised C7-C12 fragment in a highly stereoselective olefination reaction to construct the C12-C13 bond. This approach would also allow access to C26 modified analogues as we have previously described (Nicolaou et al., Angew. Chem. Int Ed. 37, 2014-2045 (1998); Nicolaou et al., Tetrahedron 54, 7127-66 (1998)). Furthermore, we wished to retain a hydroxyl group at the C-26 position until a late stage in the synthesis and thus profit from a chemo-, regio- and stereoselective Sharpless epoxidation at the C12-C13 position which we have found to be reliable (see Nicolaou et al., Angew. Chem. Int. Ed. 37, 2014-2045 (1998); Nicolaou et al., Tetrahedron 54, 7127-66 (1998)). In adopting this route we were aware that a method for subsequent deoxygenation at the C26 position (adjacent to the epoxide) would have to be developed. Finally, we hoped to apply some fine-tuning to the pivotal aldol coupling step which provides the stereocentres at C6 and C7 of the macrocycle. Previously, we have found this reaction to be somewhat capricious.

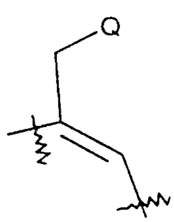
Detailed description of the invention:

The compounds according to the invention are of the formula I,

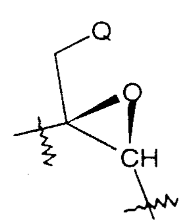


(I)

wherein the group A-D represents a group of the formula (Ia)



(Ia) or preferably of the formula (Ib)



(Ib)

wherein Q is hydroxy, iodo or preferably hydrogen.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated:

Protected hydroxy is preferably hydroxy protected by a standard protecting group. Preferably, protected hydroxy is trityloxy.

Bioactivity:
The compound(s) of the invention can be used for the treatment of a proliferative disease, especially a cancer, like cancers of the lung, especially non-small lung cell lung carcinoma, of the prostate, of the intestine, e.g. colorectal cancers, epidermoid tumors, such as head and/or neck tumors, or breast cancer, or other cancers like cancers of the bladder, pancreas or brain or melanoma, including especially the treatment of cancers that are multidrug-resistant (e.g. due to the expression of p-glycoprotein = P-gp) and/or refractory to treatment with paclitaxel (e.g. in the form of TAXOL).

The table below illustrates the ability of the compounds of the present invention to inhibit tumor cell growth as evidenced by the assays with the indicated cell lines and to block the depolymerization of microtubulin:

Biological Activity

compound	pure tubulin polymerization (%)	1A9 (parental cell line) (concentration at halfmaximal growth inhibition (nM))	PTX10 (taxol resistant cell line (concentration at halfmaximal growth inhibition (nM))	PTX22 (taxol resistant cell line (concentration at halfmaximal growth inhibition (nM))
16-desmethyl-epothilone B	65	4.0	20	8.0

Biological Evaluation:

The compounds of the present invention have valuable biological properties as described above and below.

The ability of the compounds of the present invention to block the depolymerization of microtubuli can be shown by the following assay:

Microtubule assays are carried out following literature procedures and evaluate synthesized compounds for their ability to form and stabilize microtubules. Cytotoxicity studies are

carried out as well.

The 16-desmethylepothilone is tested for its action on tubulin assembly using purified tubulin with an assay developed to amplify differences between compounds more active than Taxol. 16-Desmethylepothilone B (**1**) is found to have a high level of cytotoxic and tubulin polymerization activity, as compared to Epothilones A and B. (Lin et al. Cancer Chemother. Pharmacol. 38, 136-140 (1996); Rogan et al. Science 244, 994-996 (1984)).

Filtration Colorimetric Assay

Tubulin polymerization is determined by the filtration-colorimetric method, developed by Bollag *et Cancer Res.* **1995**, *55*, 2325-2333. Purified tubulin (1 mg/mL) is incubated at 37 °C for 30 minutes in the presence of each compound (20 mM) in MEM buffer [(100 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.75, 1 mM ethylene glycol bis(β-aminoethyl ether), *N, N, N´, N´*-tetraacetic acid, and 1 mM $MgCl_2$]; the mixture is then filtered to remove unpolymerized tubulin by using a 96-well Millipore Multiscreen Durapore hydrophilic 0.22 µm pore size filtration plate; the collected polymerized tubulin is stained with amido black solution and quantified by measuring absorbance of the dyed solution on a Molecular Devices Microplate Reader. In detail, microtubule protein (0.25 ml of 1 mg/ml) is placed into an assay tube and 2.5 µl of the test compound are added. The sample is mixed and incubated at 37 °C for 30 min. Sample (150 µl) is transferred to a well in a 96-well Millipore Multiscreen Durapore hydrophilic 0.22 µm pore size filtration plate which has previously been washed with 200 µl of MEM buffer under vacuum. The well is then washed with 200 µl of MEM buffer. To stain the trapped protein on the plate, 50 µl amido black solution [0.1% naphthol blue black (Sigma)/45% methanol/ 10% acetic acid] are added to the filter for 2 min; then the vacuum is reapplied. Two additions of 200 µl amido black destain solution (90% methanol/2% acetic acid) are added to remove unbound dye. The signal is quantitated by the method of Schaffner and Weissmann et al. *Anal. Biochem., 56*: 502-514, 1973 as follows:

200 µl of elution solution (25 mM NaOH-0.05 mM EDTA-50% ethanol) are added to the well and the solution is mixed with a pipette after 5 min. Following a 10-min incubation at room temperature, 150 µl of the elution solution are transferred to the well of a 96-well plate and the absorbance is measured on a Molecular Devices Microplate Reader.

Cytotoxicity experiments with 1A9, 1A9PTX10 (α-tubulin mutant), and 1A9PTX22 (α-tubulin mutant) cell lines can reveal the cytotoxic activity of the compounds of formula I. Like

the naturally occurring epothilones **1** and **2**, the 16-desmethyl- epothilone **B** analogue **1** shows significant activity against the altered α-tubulin-expressing cell lines 1A9PTX10 and 1A9PTX22. For compounds of the formula I, the preferred IC50 values (concentration where half-maximal growth inhibition of tumor cells is found in comparison with a control without added inhibitor of the formula I) can lie in the range of 1 to 1000 nM, preferably from 1 to 200 nM.

The ability of the compound(s) of the present invention to inhibit tumor growth can be shown by the following assays with the following cell lines:

Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening

The colorimetric cytotoxicity assay used is adapted from Skehan et al (*Journal of National Cancer Inst* 82:1107-1112, 19901). The procedure provides a rapid, sensitive, and inexpensive method for measuring the cellular protein content of adherent and suspension cultures in 96-well microtiter plates. The method is suitable for the National Cancer Institute's disease-oriented in vitro anticancer-drug discovery screen.

In particular, cultures fixed with trichloroacetic acid are stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye is removed by four washes with 1% acetic acid, and protein-bound dye is extracted with 10 mM unbuffered Tris base [tris (hydroxymethyl) aminomethane] for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. The SRB assay results are linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse subconfluence to multilayered supraconfluence. The signal-to-noise ratio at 564 nm is approximately 1.5 with 1,000 cells per well.

The SRB assay provides a calorimetric end point that is nondestructive, indefinitely stable, and visible to the naked eye. It provides a sensitive measure of drug-induced cytotoxicity. SRB fluoresces strongly with laser excitation at 488 nm and can be measured quantitatively at the single-cell level by static fluorescence cytometry (Skehan et al (*Journal of National Cancer Inst* 82:1107-1112, 19901)).

Alternatively, the efficiency of the compounds of the formula I as inhibitors of microtubuli depolymerisation can be demonstrated as follows:

Stock solutions of the test compounds are made in DMSO and stored at -20 °C. Microtubuli-protein is obtained from pig brain by two cycles of temperature dependent depolymerisation/polymerisation, as described (see Weingarten et al., Biochemistry 1974; 13: 5529-37). Working stock solutions of microtubule protein (meaning tubulin plus microtubuli-associated proteins) are stored at -70 °C. The degree of the microtubuli protein polymerisation induced by a test compound is measured essentially as known from the literature (see Lin et al., Cancer Chem. Pharm. 1996; 38:136-140). In short, 5 μl stock solution of the test compound in the twenty-fold of the desired final concentration are pre-mixed with 45 μl of water at room temperature, and the mixture is then placed on ice. An aliquot of the working stock solution of pig brain microtubuli protein is thawed quickly and then diluted to 2 mg/ml with ice-cold 2 x MEM buffer (200 ml MES, 2 mM EGTA, 2 mM $MgCl_2$, pH 6,7) (MES = 2-morpholinoethanesulfonic acid, EGTA = ethyleneglycol-bis-(2(2-aminoethyl)-tetraacetic acid). The polymerisation reaction is then started by the addition of each time 50 μl diluted microtubuli-protein to the test compound, followed by incubation of the sample in a water bath with room temperature. Then the reaction mixtures are placed in an Eppendorf microcentrifuge and incubated for additional 15 min at room temperature. The samples are then centrifuged for 20 min at 14 000 rpm at room temperature for separating polymerized from non-polymerized microtubuli protein. As indirect measure for the tubulin-polymerisation the protein concentration of the supernatant (which contains the rest of the un-polymerised, soluble microtubuli protein) is determined according to the Lowry method (DC Assay Kit, Bio-Rad Laboratories, Hercules, CA), and the optical density (OD) of the colour reaction is determined at 750 nm with a spectrometer (SpectraMax 340, Molecular Devices, Sunnyvale, CA). The differences in the OD's between samples treated with a test compound and vehicle-treated controls are compared with those of test incubations which contain 25 μM Epothilon B (positive controls). The degree of polymerisation that is induced by a test compound is expressed relatively to the positive controls (100 %). By comparison of several concentrations the EC50 (concentration where 50 % of the maximal polymerisation is found) can be determined. For compounds of the formula I the EC50 lies preferably in the range of 1 to 100, preferably 1 to 50 μM.

The efficiency against tumor cells can also be shown in the following way:

Stock solutions of the test compound of formula I 10 mM) in DMSO are prepared and stored at -20 °C. Human KB-31 and (multidrug-resistant, P-gp 170 overexpressing) KB-8511 epi-

dermoid carcinoma cells are from Dr. M. Baker, Roswell Park Memorial Institute (Buffalo, NY, USA) (for description see also Akiyama et al., Somat. Cell. Mol. Genetics 11, 117-126 (1985) und Fojo A., et al., Cancer Res. 45, 3002-3007 (1985) - KB-31 und KB-8511 both are derivatives of the KB-cell line (American Type Culture Collection) and are human epidermoid carcinoma cells. KB-31 cells can be cultivated in mono-layers using calf serum (M.A. Bioproducts), L-glutamine (Flow), penicillin (50 Einheiten/ml) und streptomycin (50 μg/ml (Flow); they then grow with a doubling rate of about 22 hours, and the relative efficiency of plating them out lies at about 60 %. KB-8511 is a variant derived from the KB-31 cell line which has been obtained by treatment cycles with colchicine, and it shows an about 40-fold relative resistance against colchicin in comparison to KB-31 cells). The cells are incubated at 37 °C in an incubator with 5 % v/v CO_2 and at 80 % relative atmospheric humidity in MEM Alpha-medium which contains ribonucleosides and desoxyribonucleosides (Gibco BRL), complemented with 10 IU Penicillin, 10 μg/ml Streptomycin and 5 % fetal calf serum. The cells are spread in an amount of 1.5×10^3 cells/well in 96-well-microtiter plates and incubated overnight. Serial dilutions of the test compounds in culture medium are added at day 1. The plates are then incubated for an additional period of four days, after which the cells are fixed using 3.3% v/v glutaraldehydem washed with water and finally stained with 0,05 % w/v methylen blue. After washing again, the stain is eluted with 3 % HCl and the optical density at 665 nm is measured with a SpectraMax 340 (Molecular Devices, Sunnyvale, CA). IC50-values are determined by mathematically fitting the data to curves using the SoftPro2.0 program (Molecular Devices, Sunnyvale, CA) and the formula

[(OD treated) - (OD start)]/[(OD control) - (OD start)] x 100.

The IC50 is defined as the concentration of a test compound at the end of the incubation period that leads to 50 % of the number of cells in comparison to controls without test compound (concentration at halfmaximal inhibition of cell growth). Compounds of the formula I preferably show here and IC50 in the range from 0.1×10^{-9} to 500×10^{-9} M, preferably between 0.2 and 50 nM.

Comparable testing can also be made with other tumor cell lines, such as A459 (lung; ATCC CCL 185), NCIH460 (lung), Colo 205 (colon; ATCC No. CCL 222) (HCT-15 (colon; ATCC CCL 225 - ATCC = American Type Culture Collection (Rockville, MD, USA)), HCT-116 (colon), Du145 (prostate; ATCC No. HTB 81; see also Cancer Res. 37, 4049-58 [1978]),

PC-3M (prostate - hormone-insensitive derivative obtained from Dr. I.J. Fidler (MD Anderson Cancer Center, Houston, TX, USA) and derived from PC-3 that is a cell line available from ATCC (ATCC CRL 1435)), MCF-7 (breast; ATCC HTB 22) or MCF-7/ADR (breast, multidrug resistant; for description see Blobe G.C.et al., J. Biol. Chem. (1983), 658-664; the cell line is highly resistant (360- to 2400-fold) to doxorubicin and Vinca alkaloids compared over MDR-7 "wild type" cells)), where similar results are obtained as with KB-31 and KB-8511 cells.

Based on these properties, the compounds of the formula I are appropriate for the treatment of proliferative diseases, such as especially tumor diseases, including also metastasis where present, for example of solid tumors, such as lung tumor, breast tumor, colorectal cancer, prostate cancer, melanoma, brain tumor, pancreas tumor, head-and-neck tumor, bladder cancer, neuroblastoma, pharyngeal tumor, or also of proliferative diseases of blood cells, such as leuemia; or further for the treatment of other diseases that respond to treatment with microtubuli depolymerisation inhibitors, such as psoriasis.

The in vivo activity of a compound of the invention can be demonstrated with the following animal model:

Female or male BALB/c *nu/nu* (nude) mice are kept under sterile conditions (10 to 12 mice per Type III cage) with free access to food and water. Mice weigh between 20 and 25 grams at the time of tumor implantation. Tumors are established by subcutaneous injection of cells (minimum 2 x 10^6 cells in 100 µl PBS or medium) in carrier mice (4-8 mice per cell line). The resulting tumors are serially passaged for a minimum of three consecutive transplantations prior to start of treatment. Tumor fragments (approx. 25 mg) are implanted s.c. into the left flank of animals with a 13-gauge trocar needle while the mice are exposed to Forene (Abbott, Switzerland) anesthesia.

Tumor growth and body weights are monitored once or twice weekly. All treatments are administered intravenously (i.v.) and are initiated when a mean tumor volume of approximately 100 to 250 mm^3 is attained, depending upon the tumor type. Tumor volumes are determined using the formula $(L \times D \times \pi)/6$ (see Cancer Chemother. Pharmacol. 24:148-154, [1989]). Treatments with epothilones of the formula I vary the dose and the frequency of

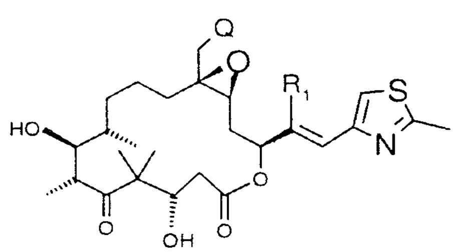
administration. Comparator agents are administered according to previously determined optimal treatment regimens. In addition to presenting changes in tu-mor volumes over the course of treatment, antitumor activity is expressed as T/C% (mean increase of tumor volumes of treated animals divided by the mean increase of tumor volu-mes of control animals multiplied by 100). Tumor regression (%) represents the smallest mean tumor volume compared to the mean tumor volume at the start of treatment, accor-ding to the formula Regression (%) = (1 -Vend/Vstart) x 100 (Vend = final mean tumor volume, Vstart = mean tumor volume at the start of treatment.

With this model, the inhibitory effect of a compound of the invention on growth e.g. of tumors derived from the following cell lines can be tested:

Human colorectal adenocarcinoma cell line HCT-15 (ATCC CCL 225) is from the American Type Culture Collection (Rockville, MD, USA), and the cells are cultivated *in vitro* as recommended by the supplier. HCT-15 is an epithelial-like cell line (Cancer Res. 39: 1020-25 [1979]) that is multi-drug resistant by virtue of over-expression of P-glycoprotein (P-gp, gp170, MDR-1 ;Anticancer Res. 11: 1309-12 [1991]; J. Biol. Chem. 264: 18031-40 [1989]; Int. J. Cancer 1991; 49: 696-703 [1991]) and glutathione-dependent resistance mechanisms (Int. J. Cancer 1991; 49: 688-95.[1991]). The Colo 205 cell line is also a human colon carcinoma cell line (ATCC No. CCL 222; see also Cancer Res. 38, 1345-55 [1978] which was isolated from ascitic fluid of a patient, dis-plays epithelial-like morphology and is generally considered to be drug-sensitive. A human androgen-independent prostate cancer cell line is used to establish subcutaneous and orthotopic models in mice. The human metastatic prostate carcinoma PC-3M is obtained from Dr. I.J. Fidler (MD Anderson Cancer Center, Houston, TX, USA) and is cultured in Ham's F12K media supplemented with 7% v/v FBS. The PC-3M cell line is the result of isolation from liver metastasis produced in nude mice subsequent to intrasplenic injection of PC-3 cells [ATCC CRL 1435; American Type Culture Collection (Rockville, MD, USA)], and they can grow in Eagle's MEM supplemented with 10% fetal bovine serum, sodium pyruvate, non-essential amino acids, L-glutamine, a two-fold vitamin solution (Gibco Laboratories, Long Island, N.Y.) and penicillin-streptomycin (Flow Laboratories, Rockville, Md.). The PC-3M cell line is hormone-insensitive (that is, it grows in the absence of androgens). The PC-3 cell line is androgen receptor negative, as is presumably the derived PC-3M cell line. PC-3 is a cell line available from ATCC (ATCC CRL

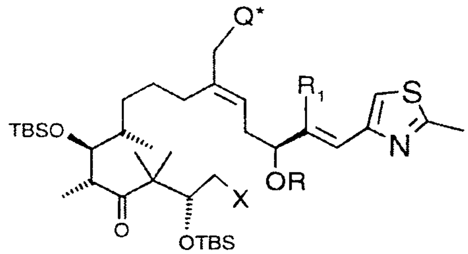
1435) and corresponds to a grade IV prostatic adenocarcinoma isolated from a 62-year-old Caucasian male; the cells exhibit low acid phosphatase and testosterone-5-α-reductase activity. The cells are near-triploid with a modal number of 62 chromosomes. No normal Y chromosomes can be detected by Q-band analysis. Human lung adenocarcinoma A549 (ATCC CCL 185; isolated as explant culture from lung carcinoma tissue from a 58-year-old Caucasian male); shows epithelial morphology and can synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway; a subtelocentric marker chromosome involving chromosome 6 and the long arm of chromosome 1 is found in all metaphases. The human breast carcinoma ZR-75-1 (ATCC CRL 1500; isolated from a malignant ascitic effusion of a 63-year-old Caucasian female with infiltrating ductal carcinoma); is of mammary epithelial origin; the cells possess receptors for estrogen and other steroid hormones and have a hypertriploid chromosome number. The human epidermal (mouth) carcinoma cell line KB-8511 (a P-gp over-expressing cell line derived from the epidermoid (mouth) KB-31 carcinoma cell line) is obtained from Dr. R.M. Baker, Roswell Park Memorial Institute (Buffalo, N.Y., USA) (for description see Akiyama et al., Somat. Cell. Mol. Genetics 11, 117-126 (1985) and Fojo A., et al., Cancer Res. 45, 3002-3007 (1985)) and is cultured as previously described (Meyer, T., et al., Int. J. Cancer 43, 851-856 (1989)). KB-8511 cells, like KB-31, are derived from the KB cell line (ATCC) and they are human epidermal carcinoma cells; KB-31 cells can be grown in mono-layer using Dulbecco's modified Eagle's medium (D-MEM) with 10% fetal calf serum (M.A. Bioproducts), L-glutamine (Flow), penicillin (50 units/ml) and streptomycin (50 mg/ml (Flow); they then grow with a doubling time of 22 h, and their relative plating efficiency is approximately 60%. KB-8511 is a cell line derived from the KB-31 cell line by use of colchicine treatment cycles; it shows about a 40-fold relative resistance against colchicine when compared with the KB-31 cells; it can be grown under the same conditions as KB-31."

The invention also relates to a method for the synthesis of a compound of the formula II,



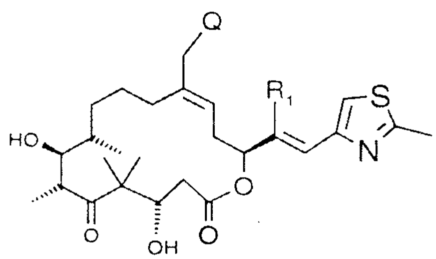
(II)

wherein Q is as described under formula I, especially hydrogen, and R_1 is methyl or preferably hydrogen, said method being characterized in that a compound of the formula III,



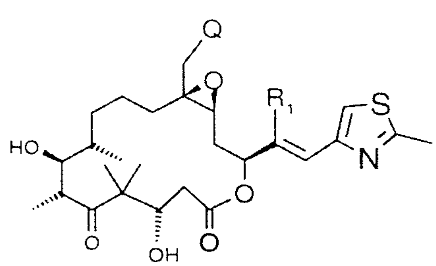
(III)

wherein TBS is tert-butyldimethylsilyl (TBS), Q* is protected hydroxy, especially trityloxy, R_1 is methyl or preferably hydrogen, X is CO_2H and R is hydrogen, is cyclised by treatment with an appropriate coupling reagent, especially first by treatment with 2,4,6-trichlorobenzoylchloride, in the presence of a tertiary nitrogen base, especially triethylamine, in an appropriate solvent, especially tetrahydrofuran, at low temperature, especially at 0 °C, and then addition of 4-dimethylaminopyridine at elevated temperature, especially at 75 °C; followed by removal of the TBS protecting groups, preferably by treatment with HF●pyridine in tetrahydrofuran at temperatures from 0 to 25 °C; and, preferably, a resulting compound of formula IV,



(IV)

wherein R_1 is as defined above, especially hydrogen, and Q is hydroxy, is converted by epoxidation into a compound of formula V (that falls under formula II),



(V)

wherein Q is hydroxy and R_1 is methyl or preferably hydrogen (in the latter case, this corresponds to a compound of formula I wherein the group A-D is of formula Ib and Q is hydroxy),

preferably by treatment with (+)-diethyl L-tartrate, $Ti(i\text{-}PrO)_4$ and t-BuOOH in methylene chloride in the presence of 4 Å molecular sieve at low temperature, especially at -30 °C; and, preferably, that compound is converted by nucleophilic substitution into a compound of formula V (that falls under formula II) wherein Q is iodo and R_1 is methyl or preferably hydrogen, (being a compound of the formula I wherein the group A-D has the formula Ib and Q is iodo), preferably by treatment with toluenesulfonyl chloride in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) in methylene chloride, and then with sodium iodide in acetone at ambient temperature, especially at about 25 °C; and preferably the resulting compound of the formula V is, by reductive deiodination, converted into a compound of the formula II wherein Q is hydrogen and R_1 is methyl or preferably hydrogen, especially by treatment with $NaBH_3CN$ and hexamethylphosphoric triamide (HMPA) at around 45 °C.

Preferably, a compound of the formula I wherein the group A-D is a group of the formula Ib and Q is hydrogen is obtained in analogy to processes that are known in the art, especially by reductively deiodinating a compound of the formula V, wherein Q is iodo and R_1 is hydrogen, in the presence of a complex hydride, especially in the presence of $NaBH_3CN$ and HMPA at around 45 °C.

A compound of the formula I wherein the group A-D is a group of the formula Ia and Q is hydrogen is preferably synthesized starting from a compound of the formula IV wherein R_1 is hydrogen and Q is hydroxy, which compound is first treated with tosyl chloride in the presence of triethylamine and 4-dimethylaminopyridine at about 0 to 25 °C, then with sodium iodide in acetone at about 25 °C and finally with $NaBH_3CN$ in the presence of HMPA at about 45 °C to yield the respective compound of formula I.

Most preferably, a compound of the formula I is obtained according to the methods described in the Examples. Most preferably, also the novel processes mentioned in the examples, as well as the single process steps or combinations of these process steps, are part of the invention.

The invention relates also to all new intermediates, especially those mentioned in the Examples.

Pharmaceutical Preparations:

The present invention also relates to the use of a compound of the formula I for the manufacture of a pharmaceutical formulation for use against a proliferative disease as defined above; or to a pharmaceutical formulation for the treatment of said proliferative disease comprising a compound of the invention and a pharmaceutically acceptable carrier.

The compounds of the formula I are called active ingredient hereinafter.

The invention relates also to pharmaceutical compositions comprising an active ingredient as defined above, for the treatment of a proliferative disease, especially as defined above, and to the preparation of pharmaceutical preparations for said treatment.

The invention relates also to a pharmaceutical composition that is suitable for administration to a warm-blooded animal, especially a human, for the treatment of a proliferative disease as defined hereinbefore, comprising an amount of an active ingredient, which is effective for the treatment of said proliferative disease, together with at least one pharmaceutically acceptable carrier. The pharmaceutical compositions according to the invention are those for enteral, such as nasal, rectal or oral, or preferably parenteral, such as intramuscular or intravenous, administration to a warm-blooded animal (human or animal), that comprise an effective dose of the pharmacologically active ingredient, alone or together with a significant amount of a pharmaceutically acceptable carrier. The dose of the active ingredient depends on the species of warm-blooded animal, the body weight, the age and the individual condition, individual pharmacokinetic data, the disease to be treated and the mode of administration; preferably, the dose is one of the preferred doses as defined below, being accommodated appropriately where pediatric treatment is intended.

The pharmaceutical compositions comprise from about 0.00002 to about 95%, especially (e.g. in the case of infusion dilutions that are ready for use) of 0.0001 to 0.02%, or (for example in case of infusion concentrates) from about 0.1% to about 95%, preferably from about 20% to about 90%, active ingredient (weight by weight, in each case). Pharmaceu-

tical compositions according to the invention may be, for example, in unit dose form, such as in the form of ampoules, vials, suppositories, dragées, tablets or capsules.

The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional dissolving, lyophilizing, mixing, granulating or confectioning processes.

Solutions of the active ingredient, and also suspensions, and especially isotonic aqueous solutions or suspensions, are preferably used, it being possible, for example in the case of lyophilized compositions that comprise the active ingredient alone or together with a pharmaceutically acceptable carrier, for example mannitol, for such solutions or suspensions to be produced prior to use. The pharmaceutical compositions may be sterilized and/or may comprise excipients, for example preservatives, stabilizers, wetting and/or emulsifying agents, solubilizers, salts for regulating the osmotic pressure and/or buffers, and are prepared in a manner known per se, for example by means of conventional dissolving or lyophilizing processes. The said solutions or suspensions may comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, carboxymethylcellulose, dextran, polyvinylpyrrolidone or gelatin.

Suspensions in oil comprise as the oil component the vegetable, synthetic or semi-synthetic oils customary for injection purposes. There may be mentioned as such especially liquid fatty acid esters that contain as the acid component a long-chained fatty acid having from 8 to 22, especially from 12 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brasidic acid or linoleic acid, if desired with the addition of anti-oxidants, for example vitamin E, betacarotene or 3,5-di-tert-butyl-4-hydroxytoluene. The alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or polyhydroxy, for example a mono-, di- or tri-hydroxy, alcohol, for example methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially glycol and glycerol.

The injection or infusion compositions are prepared in customary manner under sterile conditions; the same applies also to introducing the compositions into ampoules or vials and sealing the containers.

Preferred is an infusion formulation comprising an active ingredient and a pharmaceutically acceptable organic solvent.

The pharmaceutically acceptable organic solvent used in a formulation according to the invention may be chosen from any such organic solvent known in the art. Preferably the solvent is selected from alcohol, e.g. absolute ethanol or ethanol/water mixtures, more preferably 70% ethanol, polyethylene glycol 300, polyethylene glycol 400, polypropylene glycol or N-methylpyrrolidone, most preferably polypropylene glycol or 70% ethanol or polyethylene glycol 300.

The active ingredient may preferably be present in the formulation in a concentration of about 0.01 to about 100 mg/ml, more preferably about 0.1 to about 100 mg/ml, still more preferably about 1 to about 10 mg/ml (especially in infusion concentrates).

The active ingredient may be used as pure substances or as a mixture with another active ingredient. When used in its pure form it is preferable to employ a concentration of active ingredient of 0.01 to 100, more preferably 0.05 to 50, still more preferably 1 to 10 mg/ml (this number makes reference especially to an infusion concentrate that, before treatment, is diluted accordingly, see below).

Such formulations are conveniently stored in vials or ampoules. Typically the vials or ampoules are made from glass, e.g. borosilicate or soda-lime glass. The vials or ampoules may be of any volume conventional in the art, preferably they are of a size sufficient to accommodate 0.5 to 5 ml of formulation. The formulation is stable for periods of storage of up to 12 to 24 months at temperatures of at least 2 to 8°C.

Formulations must be diluted in an aqueous medium suitable for intravenous administration before the formulation of the active ingredient can be administered to a patient.

The infusion solution preferably must have the same or essentially the same osmotic pressure as body fluid. Accordingly, the aqueous medium preferably contains an isotonic agent which has the effect of rendering the osmotic pressure of the infusion solution the same or essentially the same as body fluid.

The isotonic agent may be selected from any of those known in the art, e.g. mannitol, dextrose, glucose and sodium chloride. Preferably the isotonic agent is glucose or sodium chloride. The isotonic agents may be used in amounts which impart to the infusion solution the same or essentially the same osmotic pressure as body fluid. The precise quantities needed can be determined by routine experimentation and will depend upon the composition of the infusion solution and the nature of the isotonic agent. Selection of a particular isotonic agent is made having regard to the properties of the active agent.

The concentration of isotonic agent in the aqueous medium will depend upon the nature of the particular isotonic agent used. When glucose is used it is preferably used in a concentration of from 1 to 5% w/v, more particularly 5% w/v. When the isotonic agent is sodium chloride it is preferably employed in amounts of up to 1% w/v, in particular 0.9% w/v.

The infusion formulation may be diluted with the aqueous medium. The amount of aqueous medium employed as a diluent is chosen according to the desired concentration of active ingredient in the infusion solution. Preferably the infusion solution is made by mixing a vial or ampoule of infusion concentrate afore-mentioned with an aqueous medium, making the volume up to between 20 ml and 200 ml, preferably between about 50 and about 100 ml, with the aqueous medium.

Infusion solutions may contain other excipients commonly employed in formulations to be administered intravenously. Excipients include antioxidants. Infusion solutions may be prepared by mixing an ampoule or vial of the formulation with the aqueous medium, e.g. a 5% w/v glucose solution in WFI or especially 0.9% sodium chloride solution in a suitable container, e.g. an infusion bag or bottle. The infusion solution, once formed, is preferably used immediately or within a short time of being formed, e.g. within 6 hours. Containers for holding the infusion solutions may be chosen from any conventional container which is non-reactive with the infusion solution. Glass containers made from those glass types afore-

mentioned are suitable although it may be preferred to use plastics containers, e.g. plastic infusion bags.

The invention also relates to a method of treatment of a warm-blooded animal, especially a human, that is in need of such treatment, especially of treatment of a proliferative disease, comprising administering a compound of the formula I, or a pharmaceutically acceptable salt thereof, to said warm-blooded animal, especially a human, in an amount that is sufficient for said treatment, especially effective against said proliferative disease.

Dosage forms may be conveniently administered intravenously in a dosage of from 0.01 mg up to 100 mg/m^2 of active ingredient, preferably from 0.1 to 20 mg/m^2 of active ingredient. The exact dosage required and the duration of administration will depend upon the seriousness of the condition and the rate of administration. The dose may be administered daily or preferably with intervals of some days or weeks, for example weekly or every 3 weeks. As the dose may be delivered intravenously, the dose received and the blood concentration can be determined accurately on the basis of known in vivo and in vitro techniques.

Pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with solid carriers, if desired granulating a resulting mixture, and processing the mixture, if desired or necessary, after the addition of appropriate excipients, into tablets, dragée cores or capsules. It is also possible for them to be incorporated into plastic carriers that allow the active ingredients to diffuse or be released in measured amounts.

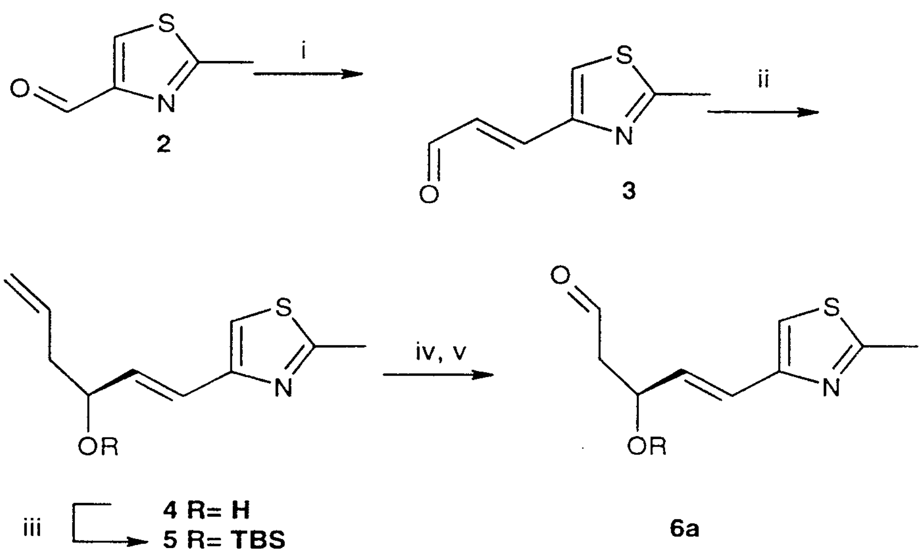
The compounds of the invention can be used alone or in combination with other pharmaceutically active substances, e.g. with other chemotherapeutics, such as classical cytostatics. In the case of combinations with an other chemotherapeutic, a fixed combination of two or more components or two or more independent formulations (e.g. in a kit of part) are prepared as described above, or the other chemotherapeutics are used in standard formulations that are marketed and known to the person of skill in the art, and the compound of the present invention and any other chemotherapeutic are administered at an interval that allows for a common, additional or preferably synergistic effect for tumor treatment.

The following examples are intended to illustrate the present invention without being intended to limit the scope of the invention.

Starting materials:

A) The thiazole fragment 6 is prepared as depicted in scheme 1 in an analogous fashion to the related epothilone B fragment with minor changes in experimental conditions. An asymmetric allylboration is the key step to introduce the stereogenic center at C15 (the ee can be shown to be equal to or more than 98 % by chiral HPLC (Chiralcel OD-H column by comparison with the racemate).

Scheme 1:

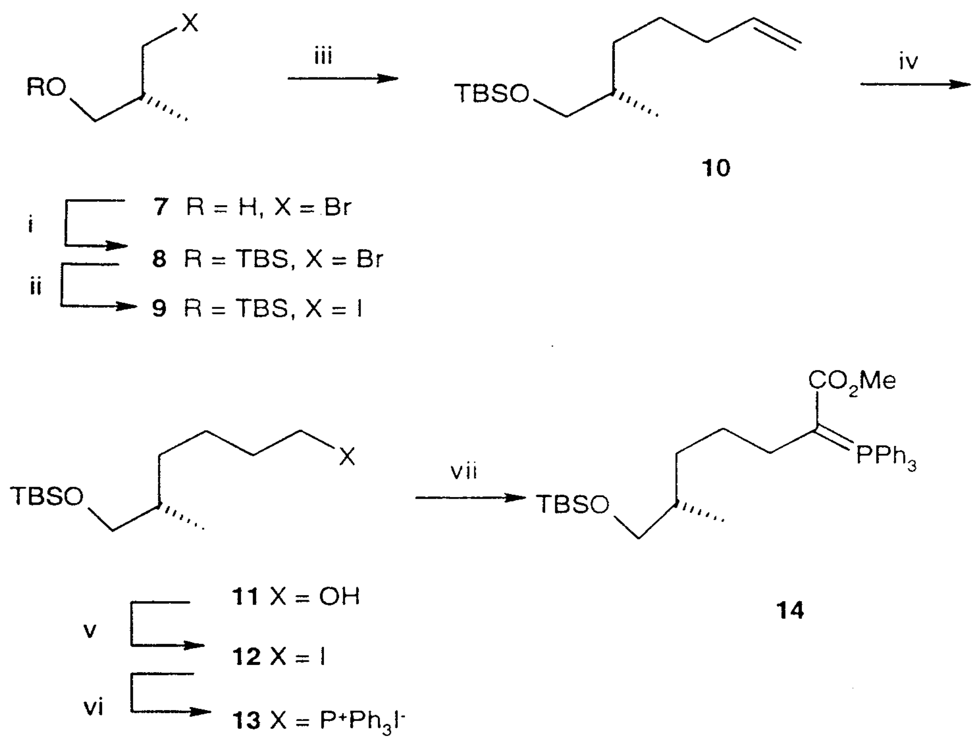


Reagents and conditions: (i) PH_3P=CHCHO, CH_2Cl_2, reflux, 12 h, 82%; (ii) (+)-Ipc_2B(allyl), CH_2Cl_2, pentane, -100 °C, 2h, 100%; (iii) TBSCl, imidazole, DMF, 0 -> 25°C, 2 h, 99%; (iv) OsO_4 (cat.), NMO, THF, t-BuOH, H_2O, 25 °C, 12 h, 68%; (v) $NaIO_4$, MeOH, H_2O, 0 -> 25°C, 1h, 89%. Ipc = isopinocampheyl, TBS = tert-butyldimethylsilyl. Physical properties of **3**: melting point (m.p.) 88-89 °C (dichloromethane/hexanes), HRMS (C_7H_7NOS) expected (M+H) 154.0327, found 154.0326; of **4**: $[\alpha]^{25}_D$ = -29.4 (c = 1.09, $CHCl_3$), HRMS ($C_{10}H_{13}NOS$) expected (M+H) 196.0796, found 196.0801; of **5**: $[\alpha]^{25}_D$ = -15.0 (c = 1.05, $CHCl_3$), HRMS ($C_{16}H_{27}NOSSi$) expected (M+Na) 310.1667, found 310.1671; of product of reaction (iv):

HRMS ($C_{16}H_{29}NO_3SSi$) expected (M+H) 344,1716, found 344.1726; and of **6a**: $[\alpha]^{25}_D = -51.9$ (c = 1.00, $CHCl_3$), HRMS ($C_{15}H_{25}NO_2SSi$) expected (M+H) 312.1454, found 312.1464.

B) The fully functionalised phosphorane **14** is prepared in short order from commercially available bromoalcohol **7** without recourse to the use of chiral auxiliaries. The reaction sequence shown in Scheme 2 includes an efficient cuprate coupling (see Tamura et al., Synthesis 1971, 303-305), and a chloroformate quench of an unstabilised ylid (see Morimoto et al., Tetrahedron 52, 10631-52 (1996)). Control of temperature during the latter reaction is critical to obtain phosphorane of good quality (gives exceedingly broad nmr spectra and no attempt to purify or rigorously characterize the intermediate is made. Quantitative yields based on mass return are obtained for the transformation of **12** to **14**. For subsequent use in Wittig reactions we assume material is pure when calculating quantities).

Scheme 2:

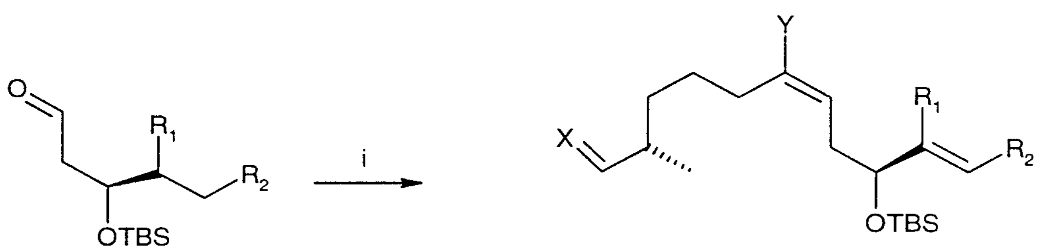


Reagents and conditions: (i) TBSCl, imidazole, DMF, 0 -> 25 °C, 1 h; (ii) NaI, acetone, reflux, 12 h, 99% (2 steps); (iii), $CH_2{=}CH(CH_2)_2MgBr$, Li_2CuCl_4 (cat.), THF, 0°C, 1h, 96%; iv (a) O_3, CH_2Cl_2, -78 °C, then PPh_3; (b) $NaBH_4$, EtOH, 0 °C, 30 min, 91 % (2 steps); (v) I_2,

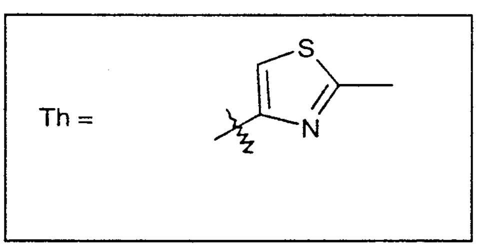
PPh_3, CH_3CN, Et_2O, 0 -> 25 °C, 1h, 100%; (vi) PPh_3, neat, 100 °C, 2h; (vii) KHMDS, THF, O°C, 30 min, then MeOCOCl, -78 °C, 3h.

C) The all-important Wittig coupling of **14** and **16** proceeds smoothly with excellent yield and selectivity (E:Z > 30:1 in all cases) to provide the coupled product **15a**, which is processed to aldehyde **21a** through a short sequence of protecting and functional group manipulations (Scheme 3). We have extended this approach to the natural epothilone B series **23b**, the 26-hydroxyepothilone B series **21b** and the highly flexible vinyl iodide series **21c**, which we have shown to be invaluable for producing heterocycle-modified epothilones (see Nicolaou et al., Angew. Chem. Int. Ed. 37, 2014-45 (1998), Nicolaou et al., Chem. Biol. 5, 365-72 (1998) and Nicolaou et al., Bioorg. Med. Chem. 1998, in press). This route also enables us to approach the highly epimerisation-prone aldehydes **21a-c** and **23** through stereochemically "safe" alcohol oxidation procedures (the aldehydes are freshly prepared and not purified prior to use).

Scheme 3:



6a, R_1 = H, R_2 = Th
6b, R_1 = Me, R_2 = Th
6c, R_1 = Me, R_2 = I



15 a, b, c X = TBSO, H; Y = CO_2Me
ii
16 a, b, c X = TBSO, H; Y = CH_2OH
iii
17 a, b, c X = TBSO, H; Y = CH_2OTr
iv
18 b X = TBSO, H; Y = CH_2Cl
vi
v
19 b X = TBSO, H; Y = CH_3
20 a, b, c X = OH, H; Y = CH_2OTr
viii
21 a, b, c X = O, Y = CH_2OTr
vii
22b X = OH, H; Y = CH_3
23b X = O, Y = CH_3
viii

Reagents and conditions: (i) **14** (1.3 equiv.), benzene, reflux, 18h, 84-93%; (ii) DIBAL-H, THF, -78°C, 3h, 71-95%; (iii) TrCl, DMAP, DMF, 70 °C, 18h, 82-95%; (iv) CCl_4, PPh_3, reflux, 18 h, 80%; (v) $LiEt_3BH$, THF, -78 °C, 1 h, 92%; (vi) HF•pyridine, pyridine, THF, 25 °C, 4h,

66-73%; (vii) CSA, MeOH, 25 °C, 1h, 95%; (viii) SO_3•pyridine, DMSO, Et_3N, THF, 0 °C, 1h. R_1 and R_2 in the (a)-series of compounds (derived from **6a**) are as defined for 6a, in the (b)-series as for **6b**, and for the (c)-series as in **6c**.

Physical properties of the intermediates:

15a: $[\alpha]^{25}_D = -7.3$ (c = 1.02, $CHCl_3$); HRMS ($C_{30}H_{55}NO_4SSi_2$) expected (M+Cs) 714.2445, found: 714.2465.

16a: $[\alpha]^{25}_D = -2.1$ (c = 1.00, $CHCl_3$); HRMS ($C_{29}H_{56}NO_3SSi_2$) expected (M+Cs) 686.2496, found 686.2518.

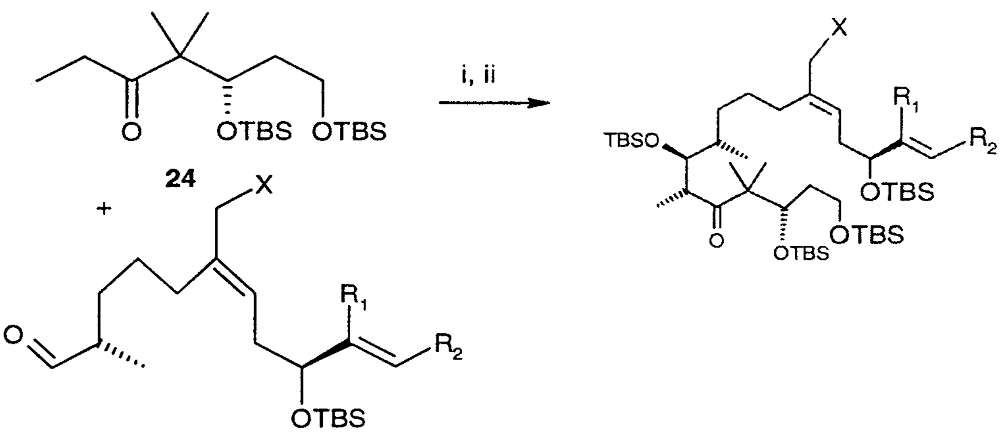
17a: $[\alpha]^{25}_D = +4.1$ (c = 1.01, $CHCl_3$), HRMS ($C_{48}H_{69}NO_3SSi_2$) expected (M+Cs) 928.3591, found 928.3621.

20a: $[\alpha]^{25}_D = +3.3$ (c = 1.21, $CHCl_3$), HRMS ($C_{42}H_{55}NO_3SSi$) expected (M+Cs) 814.2726, found 814.2754.

21a: HRMS ($C_{42}H_{53}NO_3SSi$) expected (M+Cs) 812.2570, found 812.2751.

D) We reasoned that the moderate and sometimes variable stereoselectivities obtained in the coupling of **24** with chiral aldehydes such as **21a-c** and **23b** may be due, at least in part, to the reversible nature of aldol reactions using ketone-derived lithium enolates (see Heathcock et al., in: Comprehensive Organic Synthesis, Eds. B.M. Trost et al., Pergamon, Oxford 1991, 181-235, Vol. 2; or Paterson et al., Tetrahedron Lett. 39, 8545-8 (1998)). In an attempt to alleviate such problems we perform the reaction with an excess of enolate (the unreacted ketone **24** is easily recovered by chromatography), keeping the reaction time very short and utilizing a rapid low temperature quench (acetic acid). Gratifyingly, the application of these conditions to aldehydes **21a-c** and **23b** leads to significant amelioration of stereoselectivity which is naturally accompanied by an improved yield of the desired aldol stereoisomer in each case (Scheme 4, Table 1). Intermediates **25a-d** are obtained in good overall yields after subsequent TBS-protection. Under these closely defined conditions the aldol reaction is highly reproducible and applicable to multigram quantities. These results, in terms of diastereoselectivities and yields, are at least as good as those obtained by Schinzer et al. , Synlett 1998, 861-4, in their epothilone B synthesis. Since their rather involved protecting group strategy is now avoidable, this development renders our modified approach superior.

Scheme 4:



21a, R_1 = H, R_2 = Th, X = OTr
21b, R_1 = Me, R_2 = Th, X = OTr
21c, R_1 = Me, R_2 = I, X = OTr
23b, R_1 = Me, R_2 = Th, X = H

25a, R_1 = H, R_2 = Th, X = OTr
25b, R_1 = Me, R_2 = Th, X = OTr
25c, R_1 = Me, R_2 = I, X = OTr
25d, R_1 = Me, R_2 = Th, X = H

Reagents and conditions: (i) LDA (2.4 equiv.), **24** (2.3 equiv.), THF -78 -> -40 °C, 1h, then add **21** or **23**, THF, -78 °C, 2 min, then acetic acid, -78 °C -> 0 °C (intermediate after reaction (i); (ii) TBSOTf, 2,6-lutidine, THF, -78 -> 0 °C, 1h. Th is defined as in Scheme 3.

Physical properties of the compounds:

Intermediate after reaction (i): $[\alpha]^{25}_{D}$ = -21.6 (c = 1.54, $CHCl_3$), HRMS ($C_{63}H_{99}NO_6SSi_3$) expected (M+Cs) 1214.5555, found 1214.5497.

25a: $[\alpha]^{25}_{D}$ = -17.9 (c = 0.91, $CHCl_3$), HRMS ($C_{69}H_{113}NO_6SSi_4$) expected (M+Cs) 1328.6420, found 1328.6491.

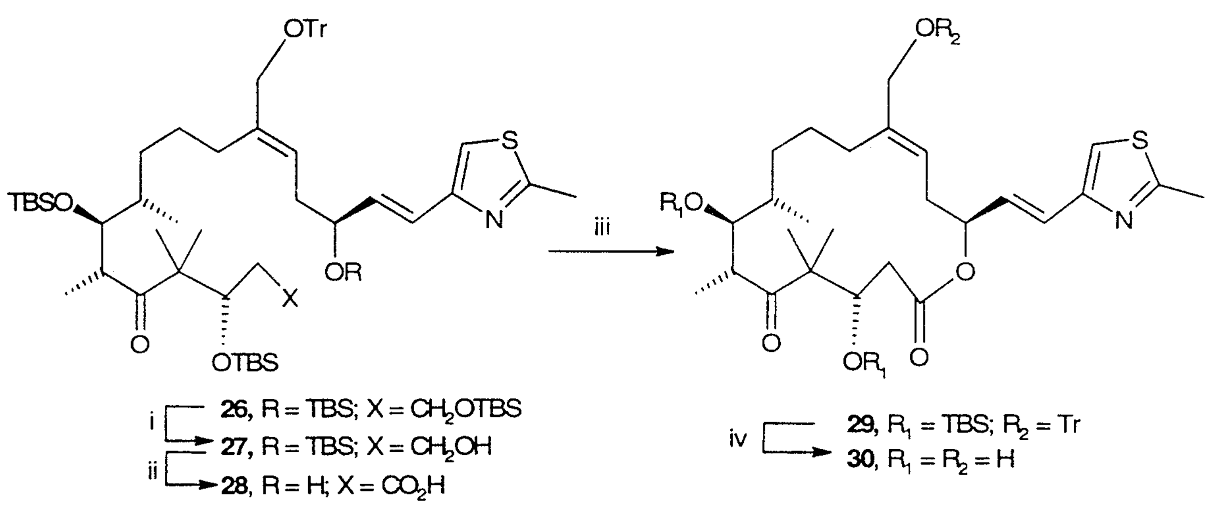
Table 1:

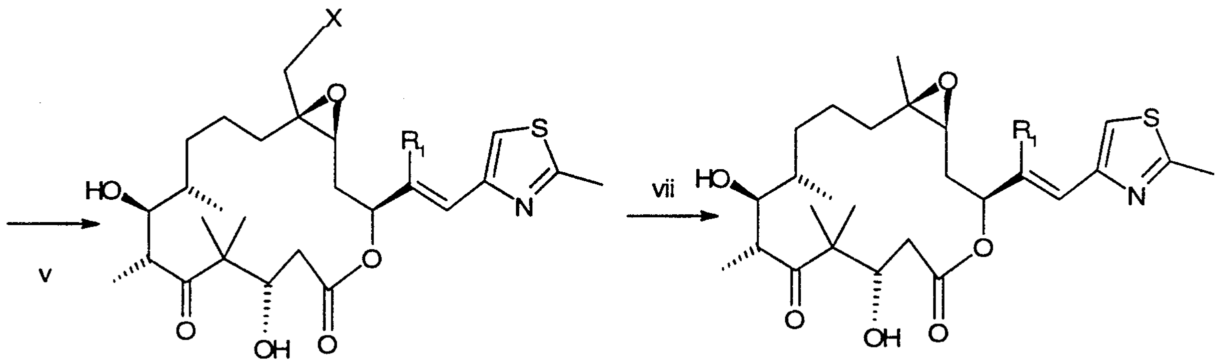
aldehyde	selectivity	yield of aldol product (%)	yield after TBS protection (2 steps) (%)
21a	≥10:1	76	72
21b	≥15:1	77	73
21c	≥10:1	79	75
23b	≥10:1	71	67

Example 1: Synthesis of 16-desmethylepothilone B (**1**) (and epothilone B (**34**))

The TBS-protected aldol product **25a** is processed through to 26-hydroxy-16-desmethylepothilone B **31** in the same fashion as the published route to 26-hydroxyepothilone B (Scheme 5). As projected, utilization of the C26 hydroxyl group in the Sharpless epoxidation facilitates stereo- and regioselective epoxidation at the C12-C13 position. For this analogue series competitive side-chain epoxidation would likely have been problematic upon use of conventional oxidants. In order to complete the synthesis, removal of the C26 hydroxyl group is required. This is achieved by initial conversion to iodide **32** followed by reductive deiodination with $NaBH_3CN$ (see Hutchins et al., Org. Chem. 42, 82-91 (1977)) to provide our desired analogue **1** in good overall yield. Furthermore, this deoxygenation sequence has also been demonstrated for the production of epothilone B (**34**) itself from our previously reported iodide **33**, Thus, all of the synthetic methodology described herein is applicable to the synthesis of epothilone B, which renders our approach now *highly selective at every step*, and significantly increases the speed of access to this and related structures.

Scheme 5:





vi: **31**, R_1 = H; X = OH → **32**, R_1 = H; X = I

33, R_1 = Me; X = I

1, R_1 = H

34, R_1 = Me

Reagents and conditions: (i) HF•pyridine, pyridine, THF, 25 °C, 3h, 87% (after 1 recycle); (ii) (a) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 30 min, then Et_3N, -78 °C -> 0 °C, 30 min; (b) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, t-butanol, H_2O, 25 °C, 2h; (c) TBAF, 25 °C, 12 h, ca. 100% (3 steps); (iii) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF, 0 °C, 1h, then add to DMAP in toluene, 75 °C, 1h, 73%; (iv) HF•pyridine, THF, 0 -> 25 °C, 24h, 73%; (v) (+)-diethyl L-tartrate, Ti(*i*-PrO)$_4$, t-BuOOH, CH_2Cl_2, 4 Å molecular sieves, -30 °C, 2h, 80%; (vi) (a) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 -> 25°C, 1h; (b) NaI, acetone, 25 °C, 91% (2 steps); (vii) $NaBH_3CN$, HMPA, 45 °C, 40 h, 67-70%. Compound **26** is identical to compound **25a**.

Physical data of compounds in Scheme 5:

27: HRMS ($C_{63}H_{99}NO_6SSi_3$) expected (M+Cs) 1214.5555, found 1214.5614.

28: HRMS ($C_{57}H_{83}NO_7SSi_2$) expected (M+Cs) 1114.4483, found 1114.4452.

29: HRMS ($C_{57}H_{81}NO_6SSi_2$) expected (M+Cs) 1096.4378, found 1096.4348.

30: HRMS ($C_{26}H_{39}NO_6S$) expected (M+H) 494.2576, found 494.2589.

31: HRMS ($C_{26}H_{39}NO_7S$) expected (M+H) 510.2525, found 510.2522.

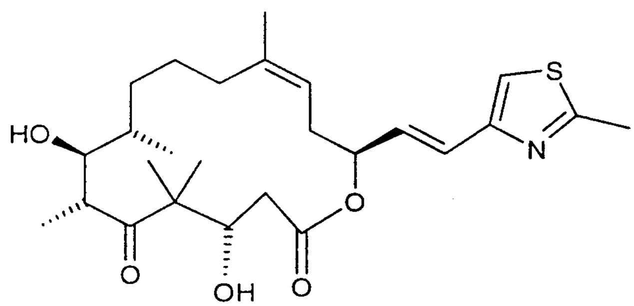
32: HRMS ($C_{26}H_{38}INO_6S$) expected (M+Cs) 752.0519, found 752.0542.

34: $[\alpha]^{25}_D$ = -34.0 (c = 2.01, $CHCl_3$, HRMS ($C_{26}H_{39}NO_6S$) expected (M+H) 494.2576, found 494.2564, IR (thin film) ν 3418, 2960, 2956, 1736, 1686, 1508, 1465, 1380, 1251, 1144, 1070, 1043, 968, 909, 733 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.96 (s, 1H), 6.65 (d, J = 15.8 Hz, 1H), 6.53 (dd, J = 15.8, 6.6 Hz, 1H), 5.69 (m, 1H), 4.10 (m, 1H), 4.00 (m, 1H), 3.32 (m, 1H), 2.89 (t, J = 6.2 Hz, 2H), 2.70 (s, 3H), 2.54 (dd, J = 9.6, 14.0 Hz, 1H), 2.47 (br s, 1H),

2.39 (dd, J = 3.7, 14.0 Hz, 1H), 2.05 (m, 1H), 1.93 (m, 1H), 1.77-1.63 (m, 3H), 1.51-1.44 (m, 2H), 1.33 (s, 3H), 1.27 (s, 3H), 1.15 (d, J = 7.0 Hz, 3H), 1.07 (s, 3H), 0.98 (d, J = 7.0 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 220.9, 170.8, 166.5, 152.1, 128.3, 125.6, 116.3, 74.8, 73.5, 72.8, 60.9, 52.4, 43.6, 39.0, 36.7, 32.9, 31.5, 30.5, 23.0, 21.1, 20.5, 19.2, 17.3, 14.3.

Example 2: Synthesis of 16-desmethyl-12,13-deoxy-epothilone B (**35**):

The title compound is synthesized from compound **30** (example 1) by reacting **30** first with (a) tosylchloride, Et_3N, DMAP, CH_2Cl_2, 0 -> 25°C, 1h; then with (b) NaI, acetone, 25 °C; and finally with $NaBH_3CN$, HMPA, 45 °C, 40 h, 61-70%, yielding the title compound of the formula



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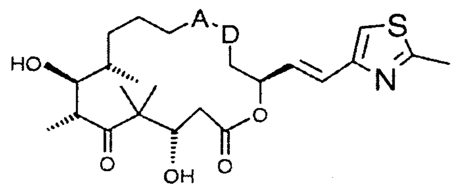
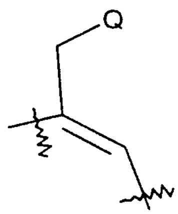
What is claimed is:

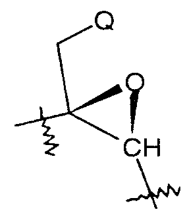
1. A compound of the formula I,



wherein the group A-D represents a group of the formula (Ia)



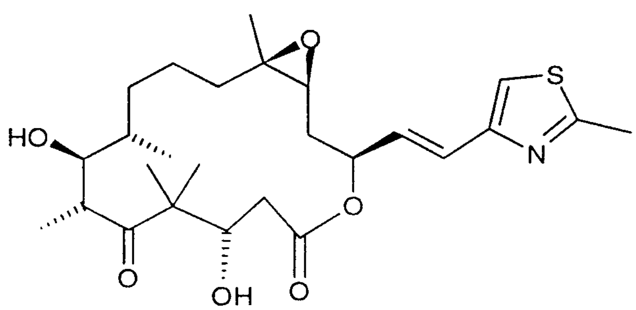
(Ia) or of the formula (Ib)



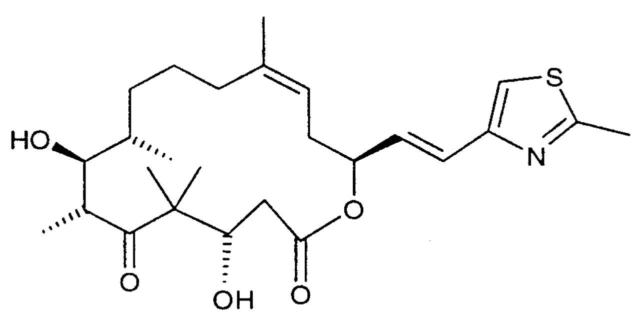
(Ib)

wherein Q is hydroxy, iodo or hydrogen.

2. A compound according to claim 1 of the formula



3. A compound according to claim 1 of the formula



4. A pharmaceutical formulation comprising a compound according to any one of claims 1 to 3 and a pharmaceutically acceptable carrier

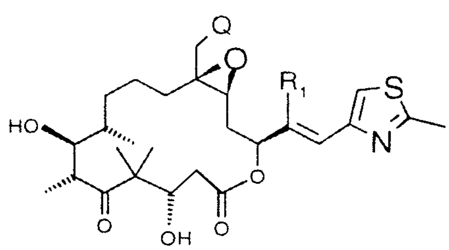
5. A compound according to any one of claims 1 to 3 for use in the treatment of a proliferative disease.

6. The use of a compound of the formula I according to any one of claims 1 to 3 for manufacture of a medicament for the treatment of a proliferative disease.

7. A method of treatment of a warm-blooded animal suffering from a proliferative disease and that is in need of such treatment, comprising administering a compound of the formula I, or a pharmaceutically acceptable salt thereof, according to claim 1 to said warm-blooded animal in an amount that is sufficient for said treatment.

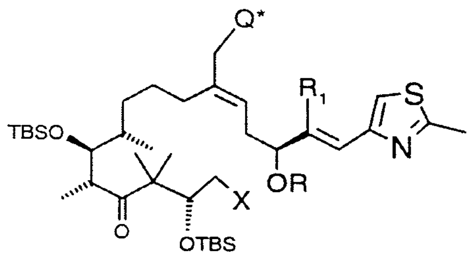
8. A pharmaceutical composition that is suitable for administration to a warm-blooded animal for the treatment of a proliferative disease, comprising an amount of an active ingredient of the formula I according to claim 1, which is effective for the treatment of said proliferative disease, together with at least one pharmaceutically acceptable carrier.

9. A method for the synthesis of a compound of the formula II,



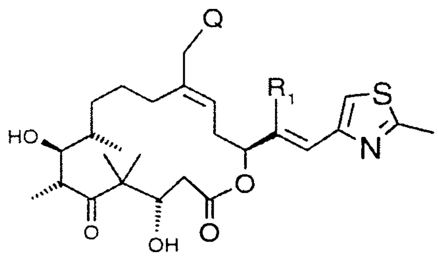
(II)

wherein Q is hydroxy, iodo or hydrogen and R_1 is methyl or hydrogen, said method being characterized in that a compound of the formula III,



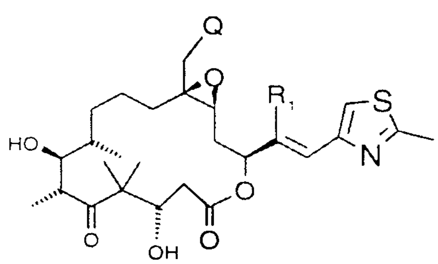
(III)

wherein TBS is tert-butyldimethylsilyl (TBS), Q* is protected hydroxy, R_1 is methyl or hydrogen, X is CO_2H and R is hydrogen, is cyclised by treatment with an appropriate coupling reagent, followed by removal of the TBS protecting groups, and a resulting compound of formula IV,



(IV)

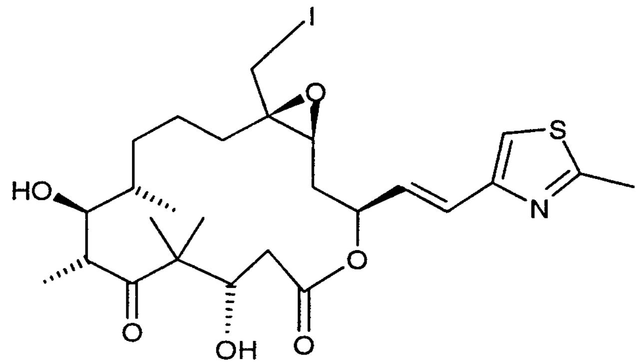
wherein R_1 is as defined above and Q is hydroxy is converted by epoxidation into a compound of formula V,



(V)

wherein Q is hydroxy and R_1 is methyl or hydrogen, and that compound is converted by nucleophilic substitution into a compound of formula V wherein Q is iodo and R_1 is methyl or hydrogen, and the resulting compound of the formula V is, by reductive deiodination, converted into a compound of the formula II wherein Q is hydrogen and R_1 is methyl or hydrogen.

10. A method for the preparation of a compound of the formula I according to claim 1 wherein the group A-D has the formula Ib and Q is hydrogen, characterized in that a compound of the formula



is reductively deiodinated.